REMARKS

Applicants have reviewed the non-final Office Action of July 10, 2006. Claims 1, 2, 3, 4, 7, 8 and 9 have been amended. Claims 7 and 36 have been cancelled. Claims 1-4, 8-13, 16-26, and 28-35 remain pending. Applicants request reconsideration of the application.

Applicants acknowledge the indication of allowable subject matter in claim 2. Claim 2 has been rewritten in independent form. Claims 3, 4, 7 and 9 have been amended to depend from previously allowed claim 2. In addition, this application now contains independent claims 1, 2, 8, 10, 20, 25, 28, 29, and 31.

A. General comments are made.

In paragraph 5 of the Office Action, the Examiner asserts the various cited references show that cyanide or azide would not kill the enzyme activity resulting in lack of anaerobic growth. These compositions include:

- anaerobe ATPase and azide (Milgrom)
- anaerobic bacterial growth in the presence of azide or cyanide (Merad and Hope)
- *E. coli* membranes and azide (Sjogren, Tarakhovskii)
- catalase and azide (Tillonen)
- glucose oxidase and azide (Midgley)

The references reportedly therefore make the following teachings:

(a) azide and cyanide allow growth of bacterial anaerobes over facultative anaerobes (Merad and Hope)

(b) most enzymes present in bacterial membrane obtained from *E. coli* still function in the presence of azide (Sjogren, Tarakhovskii)

(c) catalase in aerobic and facultative bacteria would be inhibited by azide (Tillonen)

As a result, combining bacterial membrane fragments with azide is believed to be *prima facie* obvious by the Examiner.

In response, Applicants submit that the cited references do not teach what the Examiner construes them to teach. It is important to note that *which* enzyme is inhibited

matters. Milgrom, Merad, and Hope do not aid the Examiner's case because these references disclose that azide does not inhibit anaerobic enzymes. Applicants desire the growth of anaerobes and agree that azide does not inhibit their enzymatic activity. Anaerobes are unaffected by azide because they lack the electron transport system that is inhibited by azide. Applicants agree with teaching (a) given above.

Similarly, Tillonen's teaching that catalase in aerobic and facultative bacteria is inhibited by azide supports the growth of anaerobes in azide. This teaching also suggests that one in the art would not add azide to a facultative bacterium like *E. coli* if activity is desired. Applicants agree with teaching (c) given above.

Applicants do not agree with teaching (b) given above. Applicants have taken the position that it is contrary to accepted wisdom in the art to combine azide with bacterial membrane fragments containing an electron transport system and expect the electron transport system to maintain activity. The wisdom of the art is as follows:

1. Aerobic bacteria use the electron transport system, a complex of enzymes and coenzymes located in the bacterial membrane, to generate energy used for growth. See Edwin H. Battley, Energetics of Microbial Growth, Wiley 1987, ISBN 0471084921, OCLC 13946545.
2. The electron transport system functions in bacterial membrane fragments to produce anaerobic environments. Adler, U.S. Patent No. 4,476,224.
3. Cytochrome oxidase (Complex IV) is a recognized enzyme in the electron transport system that is responsible for transferring electrons to oxygen to form water. See Garrett and Grisham, Biochemistry, pp. 641-644, Saunders 1995, ISBN 0030097584, OCLC 93087782.
4. Azide blocks growth and respiration in aerobic bacteria. See Lichstein, H.C., and Soule, M.H., Studies of the Effect of Sodium Azide on Microbic Growth and Respiration, J. Bacteriology, 1944 Mar;47(3):221-30.
5. Azide blocks growth and respiration in aerobic bacteria because it inhibits cytochrome oxidase, therefore blocking the electron transport system and the reduction of oxygen to water. See Stannard, J.N., and Horecker, B.L., The In Vitro Inhibition Of Cytochrome Oxidase By Azide And Cyanide, J. Biol. Chem. 1948, 172:599-608.

6. Therefore, one knowledgeable in the art would expect that azide blocks the electron transport system even in bacterial membrane fragments. An anaerobic environment could not be formed.

To rebut this wisdom of the art, the Examiner cites Sjogren and Tarakhovskii as teaching that most enzymes in *E. coli* membrane fragments still function in the presence of azide. Sjogren and Tarakhovskii do not make such broad teachings.

Sjogren does not support the Examiner's conclusion. In the prior Office Action of November 1, 2005, the Examiner stated that Sjogren teaches that azide is only moderately inhibitory of *E. coli*, about 15%. This is an incorrect statement. Sjogren measures the survival rate of *E. coli* and also teaches how they survive.

Sjogren desires to determine the contribution of various metabolic processes to the survival of E. coli, specifically electron transport phosphorylation, ATPase complex, and the proton motive force. See the last paragraph of page 1334. On page 1335, right column, Sjogren states that sodium azide inhibits cytochrome oxidase and ATPase. Cytochrome oxidase is an enzyme that is part of the electron transport system in mitochondria. As stated in prior amendments, ATPase is not a respiratory enzyme or an oxygen-reducing enzyme. It catalyzes the reaction: $ATP + H_2O \rightarrow ADP + P + H^+$. Clearly, this reaction does not reduce oxygen to water; in fact, it is the opposite reaction.

Sjogren therefore uses azide to inhibit the ATPase complex and the electron transport system because he wants to find out how much energy is produced from the proton motive force alone. In doing so, Sjogren uses the wisdom of the prior art. He expects that adding azide to the oxygen scavenging electron transport system (cytochrome oxidase) will stop the activity of the respiratory enzymes. On page 1336, last paragraph, he concludes the proton motive force provides additional energy to help *E. coli* survive.

Sjogren therefore does not support the Examiner's statement that most enzymes in *E. coli* membrane fragments still function in the presence of azide. Indeed, he supports the Applicants' argument that one of ordinary skill in the art would not expect the electron transport system enzymes to maintain activity in the presence of azide.

Tarakhovskii was cited as teaching that the dynamic rearrangement of *E. coli* membranes during plasmolysis proceeded and was not inhibited by azide. However, Tarakhovskii does not teach a relationship between membrane ultrastructure and membrane respiratory activity. Azide can inhibit electron transport system enzymes and

block their function without affecting the membrane ultrastructure. Tarakhovskii can be interpreted, at best, as teaching that *something* is not affected by azide. However, Tarakhovskii never teaches the identity of that something. It is unreasonable to assume that the something is an electron transport system enzyme.

In paragraph 4 of the Office Action, the Examiner responded to these arguments by stating "the Examiner's prior response included evidence directed to these effects." This statement is not responsive because it does not explain how the specific teachings of Sjogren and Tarakhovskii can be construed as broadly as the Examiner has construed them. As stated in prior responses, the effect of azide on these enzymes is not predictive of the effect of azide on respiratory enzymes. Indeed, Sjogren teaches that azide inhibits a respiratory enzyme, cytochrome oxidase. The references do not teach what the Examiner construes them to teach. It is contrary to accepted wisdom in the art to combine azide with bacterial membrane fragments containing an electron transport system and expect the electron transport system to maintain activity.

The Examiner appears to have taken the position that glucose oxidase and catalase are the respiratory enzymes on the membrane fragments. Applicants take the position that glucose oxidase and catalase are not respiratory enzymes.

B. The claims are not obvious over Merad and Adler.

Claims 1, 3-4, 7-13, 16-26, and 28-36 were rejected under 35 U.S.C. 103(a) as unpatentable over Merad in view of Adler. Applicants traverse the rejection.

In the prior amendment, Applicants argued that Merad teaches that 0.1% azide inhibits the growth of facultative microbes; one of ordinary skill in the art would therefore expect that 0.1% azide would inhibit the membrane fragments of Adler. In paragraph 7 of the Office Action, the Examiner took the position that the prior art showed that azide only moderately inhibited *E. coli* growth. The membrane fragments obtained from *E. coli* comprise additional oxygen scavenging components and would still serve to scavenge oxygen.

As explained above, Sjogren does not show the effect of azide on respiratory enzymes. Tillonen shows that azide will inhibit *E. coli* by inhibiting its catalase, so aids the Applicants' argument. Applicants agree that membrane fractions comprise additional oxygen scavenging components; they are respiratory enzymes and are the basis for the

claims of the instant application. However, the statement that the additional oxygen scavenging components in the membrane fractions would continue to scavenge oxygen in the presence of azide is shown in the record only by the Applicants' specification, not by any reference cited by the Examiner. The specification shows, in paragraphs 0085-0089, the difference between intact *E. coli* and *E. coli* membrane fragments. An anaerobic environment was created using *E. coli* membrane fragments in broth tubes containing 0.1 mg/ml NaN_3. That the membrane fragments continue to scavenge oxygen is shown by the fact that anaerobes grew in the tubes (indicated by turbidity). However, the growth of intact E. coli was inhibited, as shown by the fact that there was no turbidity in the tube. This indicates that respiratory enzymes are inhibited as well.

In paragraph 9 (misnumbered 6 on page 5 of the Office Action), the Examiner took the position that paragraph 0050 of the specification defined the membrane fragments to be "biocatalytic oxygen reducing agents" which comprise glucose oxidase. Glucose oxidase is only partially inhibited by azide, as shown by Midgley (referred to as Pseudomonas by the Examiner).

In response, Applicants agree that the membrane fragments contain biocatalytic oxygen reducing agents. However, paragraph 0050 continues by saying the essence of the Oxyrase agent are the respiratory enzymes. Applicants do not agree that glucose oxidase is a respiratory enzyme. In paragraph 0062, Applicants discuss the electron transport system which reduces oxygen to water in the presence of a hydrogen donor. This defines a respiratory enzyme. Glucose oxidase, in the presence of glucose as a hydrogen donor, converts oxygen to hydrogen peroxide, not water. Also, Midgley does not use glucose oxidase; this enzyme is never discussed in the article. Midgley instead discusses a transport system for moving glucose from the outside of the cell to the inside of the cell. This transport system does not appear to oxidize glucose. In referring to Fig. 3, Applicants agree that azide inhibits the transport system; the amount of methyl α-glucoside decreases by about 83% (from 30 to 5). However, this fact does not aid the Examiner's argument because, as Applicants have previously stated, the effect of azide on one enzyme does not predict its effect on respiratory enzymes.

To summarize, the arguments of paragraphs 7 and 9 of the Office Action are directed towards whether there is a reasonable expectation of success. Applicants again state that there is no reasonable expectation of success because accepted wisdom in the

art would teach a person skilled in the art to expect the respiratory enzymes on the membrane fragments to be inhibited by azide. Neither Merad nor Adler teach that the enzymes on membrane fragments are resistant to 0.1% azide. Thus, an anaerobic environment would not be formed and there would be no reasonable expectation of recovering anaerobic microbes.

The Examiner, on the bottom of page 4 of the Office Action, cited *in re Kerkhoven* for the proposition that it is *prima facie* obvious to combine two compositions, each taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the same purpose. In response, Applicants state that *Kerkhoven* is not applicable when the prior art also teaches not to combine the two compositions. Here, the two methods of encouraging the growth of anaerobic bacteria use different mechanisms to accomplish their purpose: oxygen scavenging membrane fragments promote anaerobic growth while azide inhibits facultative competitors. Those mechanisms may counteract each other when combined. This factor is not present in *Kerkhoven*, where two detergents were combined for use as a detergent. Here, the prior art suggests that combining azide with membrane fragments would inhibit the respiratory enzymes, thus preventing an anaerobic environment from being created. As an analogy, separate medications may be useful for making the purpose of making a person healthier, but be contraindicated for concurrent use by the same person. As a result, *Kerkhoven* is not applicable here.

Applicants previously argued that there was no motivation to combine Merad with Adler, Copeland or Fung. The Examiner responded in paragraph 10 by taking the position that the motive to combine Merad with Adler or Copeland is to relieve the lab worker from working with cumbersome physical equipment. The motive to combine Merad and Fung is to materially lessen the amount of time required for completing the assay. In response, Applicants rely on the argument that there would be no reasonable expectation of success in successfully growing anaerobes from the combination of references for the reasons given above showing that accepted wisdom in the art would teach that no success should occur. This argument against reasonable expectation of success applies to each of the three combinations of references. There is a separate argument for lack of motivation to combine Merad with Fung, discussed below.

For these reasons, the claims are not obvious based on Merad and Adler. Applicants request withdrawal of the 103(a) rejection based on Merad and Adler.

C. The claims are not obvious over Merad and Copeland.

The 103(a) rejection of claims 1, 3-4, 7-13, 16-26, and 28-36 based on Merad and Copeland (US 5,830,746) was maintained. Applicants traverse the rejection.

The combination of Merad and Copeland does not render the instant claims obvious for the same reasons given above. There is no reasonable expectation of success.

Method claims 8, 29, and 31 all teach the use of an inhibitor in the liquid broth. Neither reference discloses this claim limitation; claims 8 and 29-35 are therefore non-obvious because not all claim limitations are met. MPEP § 2143.03. This argument, drawn to these specific independent claims, was presented in the last Office Action and was not responded to by the Examiner. This is a third and independent reason to allow claims 8 and 29-35 and must be addressed by the Examiner in the next Office Action.

For these reasons, Applicants request withdrawal of the 103(a) rejection based on Merad and Copeland.

D. The claims are not obvious over Merad and Fung.

The 103(a) rejection of claims 1, 3-4, 7-13, 16-26, and 28-36 based on Merad and Fung (US 5,405,773) was maintained. Applicants traverse the rejection.

The combination of Merad and Fung does not render the instant claims obvious for the same reasons given above. There is no reasonable expectation of success.

In addition, Applicants submit that there is no motivation to combine these two references because the Examiner did not weigh the suggestive power of these two references. This argument is separate from the argument of lack of reasonable expectation of success discussed above.

Fung is trying to encourage the growth of the facultative pathogen *L. monocytogenes*. See the abstract. He also states that his assay may be used against other pathogens which are generally enterobacteria. See col. 3, lines 18-30. These enterobacteria are generally facultative anaerobes. However, Merad teaches that 0.1% azide will inhibit facultative anaerobes, which are exactly the kind that Fung wants to isolate. In other words, the combination of Merad and Fung will not materially lessen the amount of time to complete the assay, which is the motivation given to combine. Therefore, Fung teaches away from adding azide and there is no motive to combine.

Method claims 8, 29, and 31 all teach the use of an inhibitor in the liquid broth. Neither reference discloses this claim limitation; claims 8 and 29-35 are therefore non-obvious because not all claim limitations are met. MPEP § 2143.03. This argument, drawn to these specific independent claims, was presented in the last Office Action and was not responded to by the Examiner. This is a third and independent reason to allow claims 8 and 29-35 and must be addressed by the Examiner in the next Office Action.

For these reasons, Applicants request withdrawal of the 103(a) rejection based on Merad and Fung.

E. The objection to claims 7 and 8 have been corrected.

The Examiner objected to claim 7, saying that it modified the term "sample", which was not part of the claimed invention. Applicants have cancelled claim 7 and request withdrawal of the objection.

The Examiner also objected to claim 8. She stated that in paragraph e, the phrase "inoculated liquid medium composition containing the azide" was unclear. Applicants have amended the phrase to remove "containing the azide". The terminology is now consistent in steps a through e of claim 8 and is clear. Applicants request withdrawal of the objection.

F. Claim 1 complies with the written description requirement.

Claim 1 and its dependent claims were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Applicants traverse the rejection.

According to the Examiner, the phrase "self-generating anaerobic medium" had no original descriptive support. Applicants have amended claim 1 to remove the portion "self-generating". This portion was introduced in a Preliminary Amendment filed January 24, 2005. Upon review, it does not appear to have been introduced in order to overcome a specific rejection. Applicants request withdrawal of the 112 rejection.

G. The claims are not anticipated by Howell.

Claims 1, 7, and 20-26 were rejected under 35 U.S.C. 102(a) as anticipated by Howell. Applicants traverse the rejection.

Howell does not disclose all claim limitations. In the section cited on page 1235, Howell perfuses a chamber with saline, 5 mM NaN_3, 1 mM 2-deoxyglucose, and 0.3 U/ml Oxyrase. Saline is not a nutrient medium because one cannot grow cells in it. Further, deoxyglucose does not appear to be a hydrogen donor. Deoxyglucose can be used to trace glucose metabolism because it is not completely metabolized as glucose is. Thus, the combination of saline with deoxyglucose cannot be considered a nutrient medium either.

Applicants request withdrawal of the 102(a) rejection over Howell.

CONCLUSION

In view of the above amendments and comments, Applicants submit the pending claims are in condition for allowance. Withdrawal of the rejections and issuance of a Notice of Allowance is requested.

In the event the Examiner considers personal contact advantageous to the disposition of this case, she is hereby authorized to call Richard M. Klein at telephone number 216-861-5582, Cleveland, Ohio.

It is believed that no fee is due in the filing of this Response. However, if any fees are required, the Examiner is authorized to charge any fees due or credit any overpayments to Deposit Account No. 06-0308.

Respectfully submitted,

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